

CLAIMS:

1. A polynucleotide comprising a sequence encoding a polypeptide that is capable of high level presentation of antigenic peptides on antigen-presenting cells, wherein the polypeptide comprises a β_2 -microglobulin molecule that is linked through its carboxyl terminal to a polypeptide stretch that allows the anchorage of the β_2 -microglobulin molecule to the cell membrane, and through its amino terminal to at least one antigenic peptide comprising a MHC class I epitope, wherein said antigenic peptide is not related to an autoimmune disease.
2. The polynucleotide of claim 1, wherein said polypeptide stretch at the β_2 -microglobulin carboxyl terminal consists of a bridge peptide which spans the whole distance to the cell membrane, said bridge peptide being linked to a sequence which can exert the required anchoring function.
3. The polynucleotide of claim 2, wherein said bridge peptide is the peptide of SEQ ID NO: 1, of the sequence: LRWEPSSQPTIPI.
4. The polynucleotide of claim 2 or 3, wherein said bridge peptide is linked to the full or partial transmembrane and/or cytoplasmic domain of a molecule selected from the group consisting of: (i) a human MHC class I molecule selected from an HLA-A, HLA-B or HLA-C molecule; (ii) a costimulatory B7.1, B7.2 OR CD40 molecule; and (iii) a signal transduction element capable of activating T cells or antigen-presenting cells.
5. The polynucleotide of claim 4, wherein said bridge peptide is linked to the transmembrane and cytoplasmic domains from the MHC class I heavy chain HLA-A2 molecule, of the SEQ ID NO: 2, of the sequence:

VGIIAGLVLF GAVITGAVVA AVMWRRKSSDRKGGSY SQAASSDSAQ
GSDVSLTACKV

6. The polynucleotide of claim 4 wherein said transduction element capable of activating T cells is selected from the group consisting of a component of T-cell receptor CD3, a B cell receptor polypeptide, and an Fc receptor polypeptide.
- 5 7. The polynucleotide of claim 6, wherein said component of T-cell receptor CD3 is the zeta (ζ) or eta (η) polypeptide.
8. The polynucleotide of claim 6, wherein said component of T-cell receptor CD3 comprises the transmembranal and cytoplasmic regions of the human CD3 ζ
10 polypeptide.
9. The polynucleotide of claim 2 or 3, wherein said bridge peptide is linked through its carboxyl terminal to a GPI-anchor sequence.
- 15 10. The polynucleotide of claim 9, wherein said GPI-anchor is a peptide of SEQ ID NO: 3, of the sequence: FTLTGLLGTLVTMGLLT.
11. The polynucleotide of any one of claims 1 to 10, wherein said at least one antigenic peptide comprising a MHC class I epitope is linked to the β_2 -
20 microglobulin amino terminal through a peptide linker.
12. The polynucleotide of claim 1, wherein said at least one antigenic peptide is at least one antigenic determinant of one sole antigen.
- 25 13. The polynucleotide of claim 11, wherein said at least one antigenic peptide is at least one antigenic determinant of each one of at least two different antigens.
14. The polynucleotide of claim 12 or 13, wherein said antigen is a tumor-associated antigen (TAA).

15. The polynucleotide of claim 14, wherein said TAA is selected from the group consisting of alpha-fetoprotein, BA-46/lactadherin, BAGE, BCR-ABL fusion protein, beta-catenin, CASP-8, CDK4, CEA, CRIPTO-1, elongation factor 2, ETV6-AML1 fusion protein, G250, GAGE, gp100, HER-2/neu, intestinal carboxyl
- 5 esterase, KIAA0205, MAGE, MART-1/Melan-A, MUC-1, N-ras, p53, PAP, PSA, PSMA, telomerase, TRP-1/gp75, TRP-2, tyrosinase, and uroplakin Ia, Ib, II and III.
16. The polynucleotide of claim 15, wherein said antigenic peptide is selected from the group consisting of:
- 10 (i) the alpha-fetoprotein peptide GVALQTMKQ (SEQ ID NO:4);
- (ii) the BAGE-1 peptide AARAVFLAL (SEQ ID NO:5);
- (iii) the BCR-ABL fusion protein peptide SSKALQRPV (SEQ ID NO:6);
- (iv) the beta-catenin peptide SYLDSGIHF (SEQ ID NO:7);
- (v) the CDK4 peptide ACDPHSGHFV (SEQ ID NO:8);
- 15 (vi) the CEA peptide YLSGANLNL (SEQ ID NO:9);
- (vii) the elongation factor 2 peptide ETVSEQSNV (SEQ ID NO:10);
- (viii) the ETV6-AML1 fusion protein peptide RIAECILGM (SEQ ID NO:11)
- (ix) the G250 peptide HLSTAFARV (SEQ ID NO:12);
- 20 (x) the GAGE-1,2,8 peptide YRPRPRRY (SEQ ID NO:13)
- (xi) the gp100 peptides KTWGQYWQV (SEQ ID NO:14),
- (A)MLGTHTMEV (SEQ ID NO:15), ITDQVPFSV (SEQ ID NO:16), YLEPGPVTA (SEQ ID NO:17), LLDGTATLRL (SEQ ID NO:18),
- VLYRYGSFSV (SEQ ID NO:19), SLADTNSLAV (SEQ ID NO:20),
- 25 RLMKQDFSV (SEQ ID NO:21), RLPRIFCSC (SEQ ID NO:22),
- LIYRRRLMK (SEQ ID NO:23), ALLAVGATK (SEQ ID NO:24),
- IALNFPQSQK (SEQ ID NO:25) and ALNFPQSQK (SEQ ID NO:26);
- (xii) the HER-2/neu peptide KIFGSLAFL (SEQ ID NO:27);
- (xiii) the intestinal carboxyl esterase peptide SPRWWPTCL (SEQ ID
- 30 NO:28);

- (xiv) the KIAA0205 peptide AEPINIQTW (SEQ ID NO:29);
- (xv) the MAGE-1 peptides EADPTGHSY (SEQ ID NO:30) and SLFRAVITK (SEQ ID NO:31);
- (xvi) the MAGE-3 peptides EVDPIGHLV (SEQ ID NO:32) and FLWGPRALV (SEQ ID NO:33);
- (xvii) the MART-1/Melan-A peptide (E)AAGIGILTV (SEQ ID NO:34);
- (xviii) the MUC-1 peptide STAPPVHNV (SEQ ID NO:35);
- (xix) the N-ras peptide ILDTAGREEY (SEQ ID NO:36);
- (xx) the p53 peptide LLGRNSFEV (SEQ ID NO:37);
- (xxi) the PSA peptides FLTPKKLQCV (SEQ ID NO:38) and VISNDVCAQV (SEQ ID NO:39);
- (xxii) the telomerase peptide ILAKFLHWL (SEQ ID NO:40);
- (xxiii) the TRP-1 peptide MSLQRQFLR (SEQ ID NO:41);
- (xxiv) the TRP-2 peptides LLGPGRPYR (SEQ ID NO:42), SVYDFFVWL (SEQ ID NO:43), and TLDSQVMSL (SEQ ID NO:44);
- (xxv) the TRP2-INT2 peptide EVISCKLIKR (SEQ ID NO:45); and
- (xxvi) the tyrosinase peptide KCDICTDEY (SEQ ID NO:46).

17. The polynucleotide of any one of claims 14 to 16, wherein said at least one antigenic peptide is at least one antigenic determinant of one sole tumor-associated antigen.

18. The polynucleotide of claim 17, wherein said at least one antigenic peptide is at least one HLA-A2 binding peptide and at least one HLA-A3 binding peptide derived from the melanoma-associated antigen gp100.

19. The polynucleotide of claim 18, wherein said at least one HLA-A2 binding peptide derived from gp100 is selected from the group consisting of SEQ ID NO: 14, 15, 16, 17, 18, 19, 20, 21 and 22, and said at least one gp100 HLA-A3 binding peptide is selected from the group consisting of SEQ ID NO: 23, 24, 25 and 26.

20. The polynucleotide of any one of claims 14 to 16, wherein said at least one antigenic peptide is at least one antigenic determinant of each one of at least two different tumor-associated antigens.

5 21. The polynucleotide of claim 20, wherein said at least one antigenic peptide is at least one HLA-A2 binding peptide derived from each one of the melanoma associated antigens gp100 and Melan-A/MART-1.

10 22. The polynucleotide of claim 21, wherein said at least one antigenic peptide is at least one HLA-A3-restricted gp100 and at least one HLA-A2-restricted Melan-A/MART-1 peptide.

15 23. The polynucleotide of claim 12 or 13, wherein said antigen is an antigen from a pathogen selected from the group consisting of a bacterial, viral, fungal and parasite antigen.

24. The polynucleotide of claim 23 wherein the antigen is a viral antigen.

20 25. The polynucleotide of claim 24 wherein the viral antigen is an HIV protein selected from the group consisting of the HIV-1 regulatory proteins Tat and Rev and the HIV envelope protein, in which case the antigenic peptide derived therefrom has the sequence RGPGRAFVTI (SEQ ID NO:47).

25 26. The polynucleotide of claim 11, wherein said at least one antigenic peptide is at least one idiotypic peptide expressed by autoreactive T lymphocytes.

30 27. The polynucleotide of claim 26, wherein said at least one idiotypic peptide is derived from a CDR (complementarity-determining region) sequence of an immunoglobulin or of a TCR chain, optionally containing said CDR flanking segments.

28. The polynucleotide of claim 27, wherein said CDR is the CDR3 of an immunoglobulin or of a TCR chain.
- 5 29. The polynucleotide of any one of claims 1 to 28 that is an expression vector.
30. An expression vector comprising a polynucleotide according to any one of claims 1 to 28.
- 10 31. A recombinant viral vector of claim 30.
32. An antigen-presenting cell transfected with a polynucleotide comprising a sequence encoding a polypeptide comprising a β_2 -microglobulin molecule that is linked through its carboxyl terminal to a polypeptide stretch that allows the anchorage of the β_2 -microglobulin molecule to the cell membrane, and through its amino terminal to at least one antigenic peptide comprising a MHC class I epitope.
- 15 33. The antigen-presenting cell of claim 32 selected from the group consisting of a dendritic cell, a macrophage, a B cell and a fibroblast.
- 20 34. The antigen-presenting cell of claim 32 or 33 wherein said antigenic peptide is a peptide not related to an autoimmune disease.
35. The antigen-presenting cell of claim 34, wherein said antigenic peptide is at least one peptide derived from at least one TAA.
- 25 36. The antigen-presenting cell of claim 34, wherein said antigenic peptide is at least one peptide derived from an antigen from a pathogen selected from the group consisting of a bacterial, a viral, a fungal and a parasite antigen.

37. A DNA vaccine comprising a polynucleotide of any one of claims 1 to 28 or an expression vector of claim 30 or 31.

38. The DNA vaccine of claim 37 for prevention or treatment of cancer wherein said polynucleotide is a polynucleotide of any one of claims 14 to 22.

39. The DNA vaccine of claim 37 for prevention or treatment of a disease caused by a pathogenic organism wherein said polynucleotide is a polynucleotide of any one of claims 23 to 25.

40. A cellular vaccine, which comprises an antigen presenting cell of claim 32.

41. The cellular vaccine of claim 39 wherein the antigen presenting cell is selected from the group consisting of a dendritic cell, a macrophage, a B cell and a fibroblast.

42. The cellular vaccine of claim 41, wherein the at least one antigenic peptide presented by the antigen presenting cell is a peptide not related to an autoimmune disease.

43. The cellular vaccine of claim 42 for prevention or treatment of cancer wherein the antigen presenting cell presents at least one peptide derived from at least one tumor associated antigen.

44. The cellular vaccine of claim 42 for prevention or treatment of a disease caused by a pathogenic organism wherein the antigen presenting cell presents at least one peptide derived from a pathogenic antigen.

45. A cellular vaccine for the prevention or treatment of cancer comprising antigen presenting cells which express a polypeptide consisting of β_2 -microglobulin linked through its carboxyl terminal to a polypeptide stretch that allows the anchorage of the β_2 -microglobulin molecule to a cell membrane, wherein said cells

have been pulsed with at least one antigenic peptide derived from at least one tumor associated antigen.

46. A cellular vaccine for treatment of cancer comprising tumor cells transfected with a polynucleotide comprising a sequence encoding a polypeptide comprising a β_2 -microglobulin molecule that is linked through its carboxyl terminal to a polypeptide stretch that allows the anchorage of the β_2 -microglobulin molecule to the cell membrane.

47. A method of immunizing a mammal against a tumor-associated antigen comprising the step of immunizing the mammal with a cellular vaccine of any one of claims 43, 45 or 46.

48. A method of immunizing a mammal against a disease caused by a pathogenic organism comprising the step of immunizing the mammal with a cellular vaccine of claim 44.

49. A pharmaceutical composition comprising as an active ingredient at least one polynucleotide of any one of claims 1 to 29 or an expression vector of claim 30 or 31, and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49 wherein the polynucleotide comprises a sequence encoding a polypeptide comprising at least one antigenic peptide derived from at least one tumor associated antigen.

51. The pharmaceutical composition of claim 49 wherein the polynucleotide comprises a sequence encoding a polypeptide comprising at least one antigenic peptide derived from a pathogenic antigen.

52. A pharmaceutical composition comprising as an active ingredient at least one antigen presenting cell of any one of claims 32 to 36, and a pharmaceutically acceptable carrier.